

## Chapter 35

### Coral spawning at the Birch Aquarium at Scripps: Observations on timing and behavior

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#### ABSTRACT

The spawning and reproductive behavior of an *Acropora valida* (Dana, 1846) colony [50 cm diameter], maintained at the Birch Aquarium at Scripps in La Jolla, California USA (Lat. 32°52.0'N), was recorded from 1999 to 2003. With the intent to predict the colony's spawning, its polyps were dissected periodically to determine the level of gamete maturity. This activity, paired with environmental cues such as water temperature and the lunar phase, was used to predict spawning events.

The *A. valida* colony shifted its spawning time from that of its place of origin, Fiji (exact location not verified) to that of its conspecifics in the northern hemisphere, namely Hawaii and Taiwan (Kenyon, 1992, Dai *et al.*, 1992). Spawning occurred 3 to 4 weeks after a rise in water temperature, typically June through July when room temperatures rose due to a rise in outdoor temperature. In two of the 5 years, spawning was observed to be split between two consecutive nights. Spawning was observed at approximately 21:45 hours and spawning three hours after sunset (22:30 hours). Spawning of egg-sperm bundles was initiated by polyps on lower (older) branches and proceeded to polyps on higher (younger) branches, lasting approximately 45 minutes. Polyps at branch tips were also observed spawning. Self-fertilization experiments produced no viable larvae and it is unknown whether fertilization took place at all. These observations, and more specifically the histological examination of ovary development (orange-pink pigmentation) demonstrated that predictions could be made within 7-10 days of actual spawn date, allowing opportunities for propagation efforts and research studies. This paper offers a rare observation of consecutive *Acropora* spawns in captivity.

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#### INTRODUCTION

Evidence of a coral spawning was found the morning of July 5, 1999 in a tank containing corals from the Indo-Pacific, at the Birch Aquarium at Scripps (BAS). Yellow froth in the reservoir and bright orange-pink eggs trapped in the filter socks confirmed that spawning had taken place the night of July 4, 1999, 5 nights after a full moon. On the advice of Dr. Nancy Knowlton (Scripps Institution of Oceanography, SIO) that some corals split their spawn between two consecutive nights, the author observed the Indo-Pacific reef exhibit the night of July 5, 1999. At approximately 22:30 hours, a few egg-sperm bundles became visible from an *Acropora valida* (Dana, 1846) colony that had resided at the BAS for 4.3 years. Most of the

spawn was videotaped (see Figures 1 and 2). At that time it was unclear whether it was this colony that had spawned the night before.

For the next 4 years, the *A. valida* colony was monitored more closely, for signs of spawning readiness by performing polyp dissections and examining ovary development. Cues normally associated with coral spawns, such as water temperature change, the lunar phase and possibly increasing day length (Harrison and Wallace, 1990), were also taken into consideration to help predict spawning dates.

Witnessing the release of egg-sperm bundles allowed observation of spawning behavior



Figure 1: Spawning of *Acropora valida* on the night of July 5, 1999. Note: video camera date incorrect.

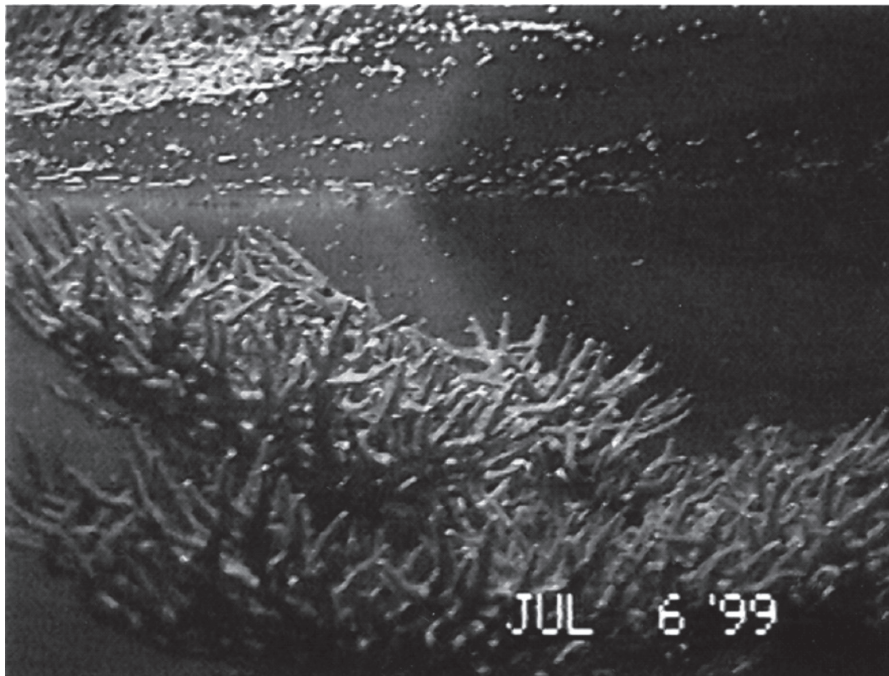


Figure 2: Egg-sperm bundles floating at water's surface, after the *Acropora valida* colony's spawning event, on the night of July 5, 1999. Note: video camera date incorrect.

and collection of gametes for propagation experiments. In total, 5 consecutive years of spawning dates were recorded. Although there are some trends that became apparent from the data gathered, we were unable to predict the exact night of a spawn. However, spawns of this *A. valida* colony could be predicted to within 10 days. Rising tank water temperature, from an average of 25 °C to a maximum of 28.5 °C would indicate that that

gamete development/maturity was underway. Polyp dissection, during the period of water temperature rise, with close attention to ovary pigmentation provided a time frame for predicting a spawning event. Specifically, bright orange-pink pigmentation of the ovaries was a sign that spawning would occur within 10 days. Given this ability, captive coral spawning events may provide opportunities for gamete collection and research.

## MATERIALS AND METHODS

### *Colony history*

The *A. valida* colony observed in this study was purchased in February 1995, from a Los Angeles wholesaler. The colony originated from Fiji (Votava/contact at wholesaler, personal communication) and was approximately 11 cm in diameter and 9 cm tall. The colony was identified as *A. valida* (Fukami and Hough, pers. com.) based on visual observation of the live colony. At the time of the first observed spawning (July, 1999), this colony had resided at the BAS for 4.3 years and attained a size of 50 cm in diameter.

### *Polyp dissections*

Observing coloration in coral eggs is a useful technique for predicting coral spawns. Polyp dissections were performed in order to determine spawn readiness and whether a spawning event took place. Fragments of *A. valida*, approximately 5 cm long, were cut with stainless steel bone cutters along the branch's axis and observed under dissecting scope. Ovaries within polyps were examined for color change as an indication of spawn readiness. This was performed monthly and weekly as the suspected spawn date approached. The absence of eggs in polyps and their presence in filter socks were used as evidence that *A. valida* had spawned.

### *Spawning behavior*

The spawn of 1999 was observed and videotaped (Sony Model No. CCD-TRV12, Video 8, Sony Corp. Japan). Portions of the spawn of 2001 and 2003 were also captured on film (Sony mini DV, Model No. DCR-PC110, Sony Corp, Japan). When spawns were observed live, the tank's circulation pump was turned off and one tank metal halide bulb was turned on in order to view and videotape.

### *Cues*

Tank temperatures were not manipulated for the 1999 spawns. Yearly mean tank water temperature was 25.5 °C. Rising outdoor temperatures increased room temperatures, which in turn, gradually raised tank water temperature to 27 °C – 29.5 °C. From 2000 to 2003 tank temperatures were monitored closely and allowed to rise a few weeks before June/July. Records of the natural lunar phase and sunsets in San Diego, CA were obtained from the computer program "Starry Night" (Starry

Night-Backyard Version 3.1, Imaginova Corp. USA). See Appendix I for details regarding lighting, moonlight, etc.

### *Fertilization experiments*

Egg-sperm bundles from *A. valida* spawns of 1999, 2000 and 2003 were collected with a small glass bowl. Sperm were separated from eggs by filtering through 100 µm Nitex screen (Nitex, Tobler, Ernst and Traber, Inc. NY, USA). A diluted solution of sperm was reintroduced to the eggs, which were placed into 4 L containers filled with seawater from the *A. valida* colony's tank. The 4 L containers were partially submerged into a reserve coral tank (1,135 L) with the same water temperature. Each container was gently aerated using airline tubing. Small pieces of live rock were added to the containers as substrate for planula to settle on.

## RESULTS

### *Polyp dissections*

Oocyte pigmentation was white months before a spawn. Approximately 3-4 weeks prior to spawning a gradual change from white to yellow pigmentation was observed. This change in ovary pigmentation was close to the point at which water temperature rose from the average yearly temperature of 25.5 °C. Ovaries were bright, orange-pink days before a spawn (within 10 days). Six to eight oocytes per polyp were observed.

### *Timing*

Spawn dates were recorded (see Figure 3 and Table 1) and correlated to water temperature and the lunar phase. Spawns occurred late June through July except for the spawn of 2001, which took place in April. For spawning events that were observed (1999, 2002, 2003), setting behavior occurred at about 21:30-21:45 h. The spawning of 5 July 1999 was initiated at 22:30, three hours after sunset and lasted nearly 45 minutes.

### *Spawning behavior*

The entire spawning event of 5 July 1999 was observed. Spawning began with polyps on lower (older) branches and gradually proceeded with those on higher (younger) branches (see Figure 4). The very beginning of the 2002 spawn was viewed. Setting behavior was visible from 21:30-21:45. The end of the 2003 spawn occurred close to 23:00 hours, which is close to the time



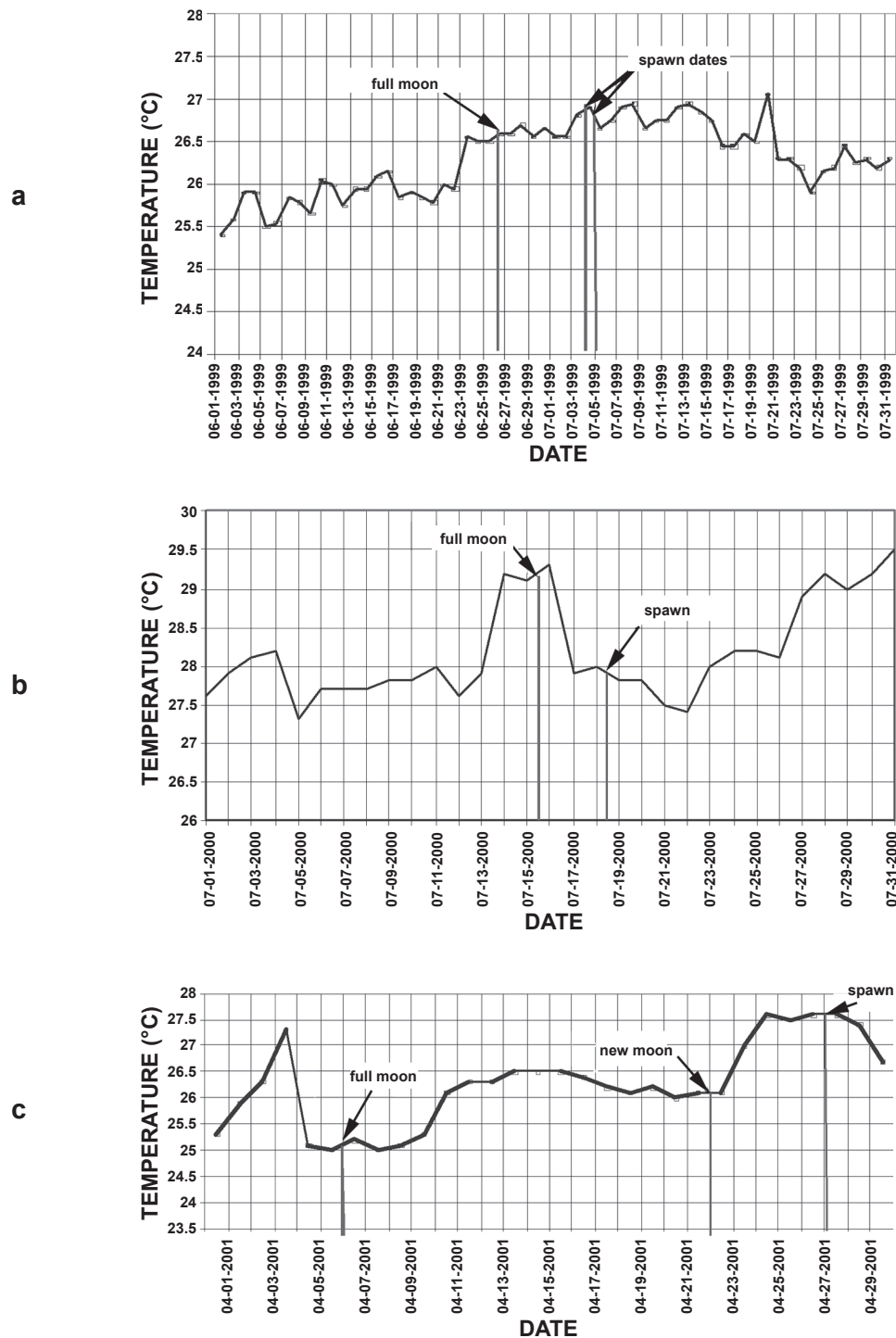


Figure 3a-c: Graphs of *Acropora valida* spawns, 1999-2003 showing spawning dates in relation to water temperature and the lunar phase.

when the spawn of 1999 ended.

Numerous axial polyps were observed spawning (see Figure 4). Observations from two of the five spawning years indicated that *A. valida* splits its spawn between two consecutive nights. While performing polyp dissections in 2002, it was observed that some polyps, within the colony, contained ripe eggs (bright orange-pink) while others immature (lightly pigmented) eggs.

#### Fertilization experiments

Three attempts, in three different years were made at self-fertilizing eggs and sperm of *A. valida*. None were successful in producing planula larvae. In 1999, the author improvised the techniques used to fertilize. In 2002 and 2003 assistance was provided by David Kline (SIO), who had field and lab experience, but these attempts did not produce viable planulae.

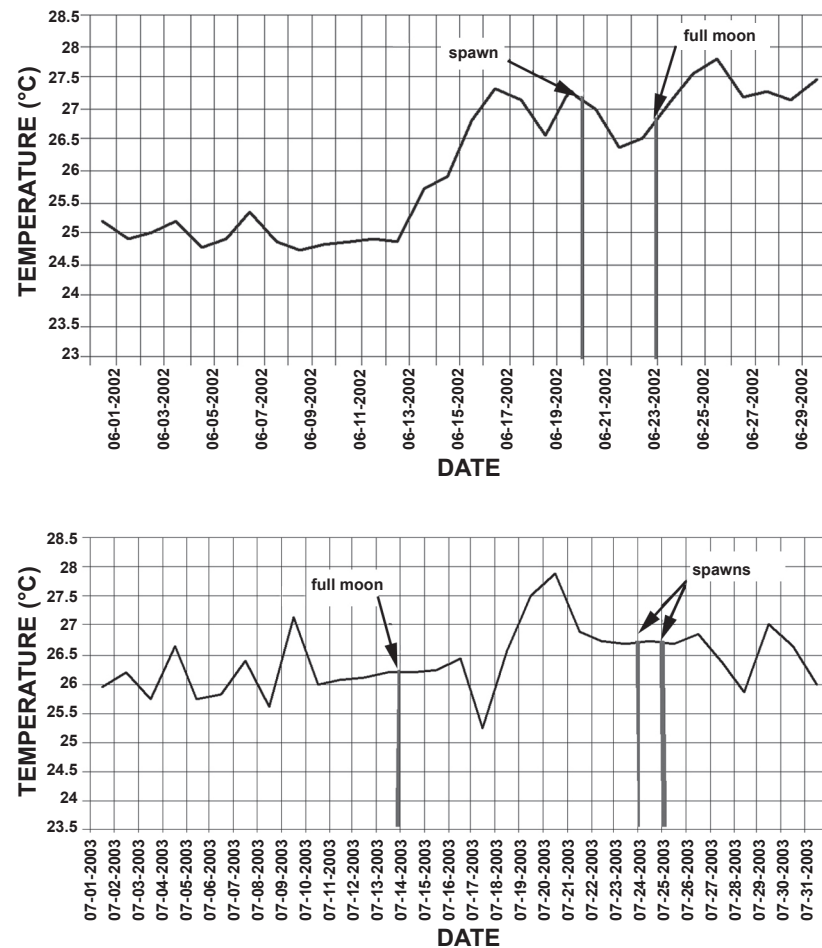


Figure 3d-e: Graphs of *Acropora valida* spawns, 1999-2003 showing spawning dates in relation to water temperature and the lunar phase.

Table 1: Summary of Spawning Records for *Acropora valida* at the BAS, 1999 -2003

Spawning date	Water temp.(°C)	Days After(+) Days Before(-) Full moon	Observed/Inferred
4 July 1999	26.8	+5	Inferred
5 July 1999	26.7	+6	Observed
19 or 20 July 2000	27.8	+3 or +4	Inferred
28 April 2001	27.6	- 9	Inferred
21 June 2002	27.0	- 3	Observed
24 July 2003	27.8	+11	Observed
25 July 2003	27.9	+12	Observed

## DISCUSSION

### Overview

The *A. valida* colony at BAS shifted its spawning time by up to six months compared to that of its conspecifics in Fiji (Babcock, pers. com.) such that the colony mirrored the spawns of its conspecifics in the northern hemisphere (Kenyon, 1992, Bermas, *et al.*, 1992, Dai *et al.*,

1992). Rising water temperature of about 2.5 °C seemed to be an important proximate cue and is an easily manipulated factor in timing a coral spawning. Orange-pink eggs signified that spawning was going to take place within ten days. Of the three observed years, setting (21:30-21:45 hours) and spawning times (22:30-23:15 hours) seem to be fairly consistent. Although the exact day of a spawn could not be

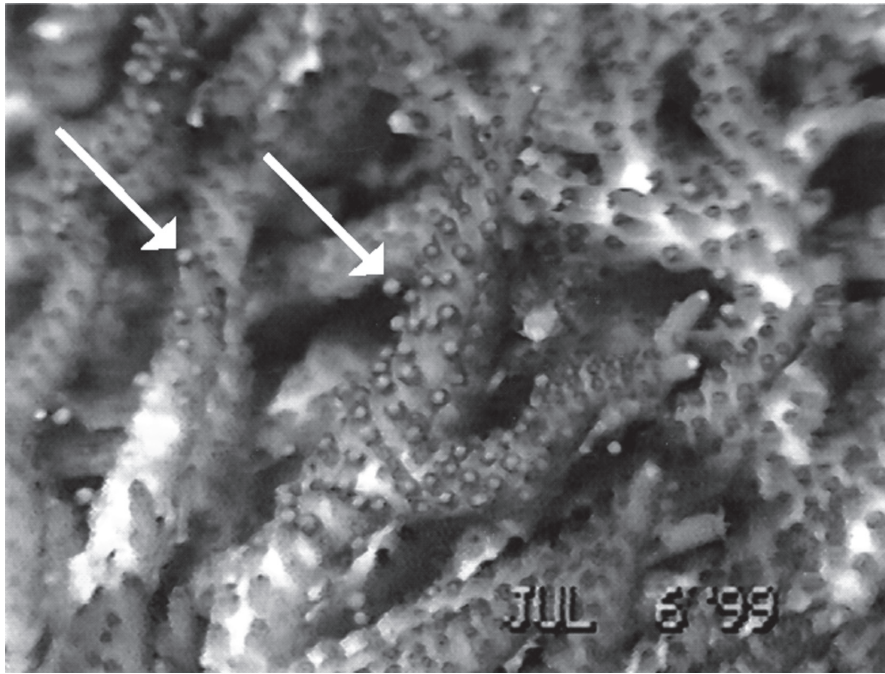


Figure 4. The *Acropora valida* colony's spawn was initiated from polyps on lower branches with polyps located at the top branches spawning last. This photo represents the end of the spawn as these polyps at the top of the colony are set to spawn (right arrow). Left arrow pointing to axial polyp "set" to spawn.

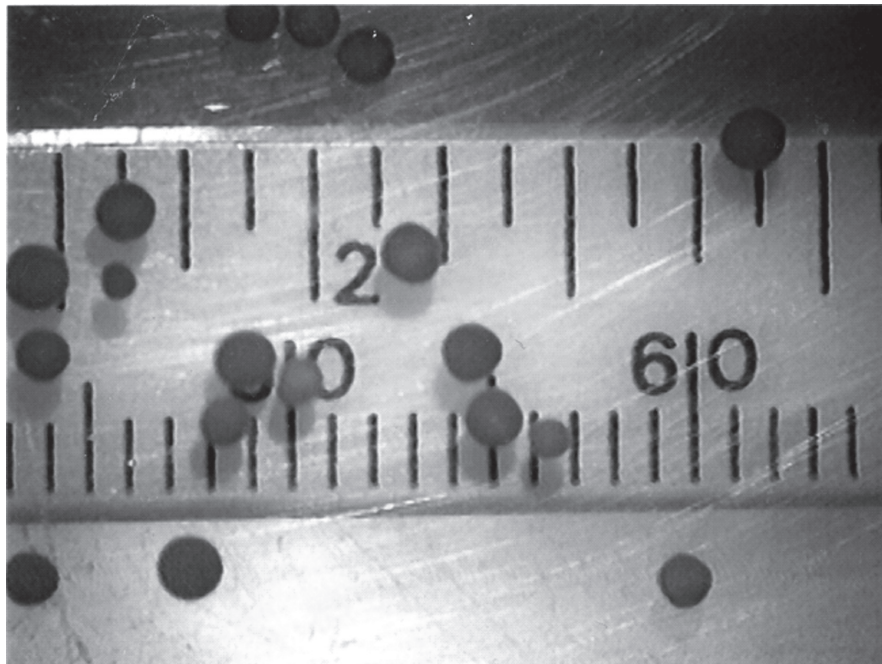


Figure 5: Egg-sperm bundles collected from the *Acropora valida* colony at the BAS. Self-fertilization experiments were unsuccessful in producing planula larvae.

predicted, the setting time and hour of spawning seem quite predictable. Paul Hough (pers. com.) stated that *A. valida*, normally spawn at 21:30 hours near Townsville, Australia, and in the Abrolhos Islands, Australia, spawning took place from 21:05-22:30 hours (Babcock *et al.*, 1994). Manipulating cues such as water temperature, photoperiod and the lunar phase are within the aquarist's means. Still, a better understanding

of captive coral spawning in terms of timing and the relationship to natural and/or artificial cues is necessary to accurately predict captive spawning events. Furthermore, this knowledge will have to be gained on a species-by-species basis. Understanding one coral's response to cues may not help though. For example, it is interesting to note that an unidentified *Acropora* sp. colony also from Fiji, and in the same tank,

did not spawn when *A. valida* spawned. In fact, it spawned two to three months later, as the water temperature gradually decreased. A *Turbinaria reniformis* colony was also observed spawning, in the same tank. It spawned five days after the full moon (June 22, 2000) at approximately 19:30 hours (dusk). In the Great Barrier Reef (Willis *et al.*, 1985) found that mass spawning times of corals differed from inshore versus offshore reefs. So, although we may be able to deduce the cues necessary for one particular species of coral, the same cues may not influence other species, even those of the same genera and from the same locality. It is hoped that with continued observations and a better understanding of how natural and artificial cues influence coral spawning, aquarists can better manipulate tank conditions to make full use of the opportunities spawning events may provide.

#### **Ovary pigmentation**

Ovary pigmentation was the greatest factor in helping to predict a spawn. When ovaries displayed a deep orange-pink pigmentation, a spawn was to occur within a week to 10 days. Ovaries of *A. valida* from the Northwestern Hawaiian Islands display a similar color (Kenyon, 1992).

#### **Water temperature**

Water temperature seemed to be the most important cue in preparation for a spawn. This is consistent with the literature (Dubinsky, 1999). The spawn of April 2001 was early and was due to a premature and unchecked rise in water temperature. This observation shows that the timing of a spawn can be manipulated to a certain degree. The sudden increase in water temperature seemed to accelerate the gametogenic cycle. Wallace (2001) states that gametogenic cycles for acroporids can be 7–10 months long and the spawn of 2001 still falls within this range. All other spawns occurred late spring or early summer when mean water temperature (25.5 °C) gradually rose up to 27.5 °C–29.5 °C. Kenyon (1992) observed the water temperature of 29.5 °C the day before *A. valida* colonies spawned in the wild.

#### **Day length**

Tank lights were never manipulated to change photoperiod (see Appendix I). However, there may have been some influence from filtered sunlight coming through the skylight. Longer days during spring and summer would light up

the room, where the tank was located, before tank lights came on and extend sunset past the time when overhead light were turned off.

#### **Lunar phase**

The spawns of 1999 and 2000 occurred three to six days after the natural full moon, which is consistent with conspecifics from different localities (Wallace, 2000). However the spawning events of 2001 and 2002 occurred days before a full moon. It is unclear why this “early” spawning occurred. One possibility is that the artificial moonlight may have interfered with the natural lunar phase. The influence of natural moonlight is suspect though. On numerous nights while waiting for *A. valida* to spawn, little to no moonlight was observed coming through the skylight. This was either due to the position of the moon or because of heavy fog (“marine layer”). The interval between sunset and the timing of the lunar phase may be an important cue to set the exact time of spawn (Knowlton, pers. com.).

It was observed on the night of the 2002 spawn that the fluorescent room lights (2x 40 W fluorescent bulbs) approximately 3 m distance from the tank, were left on. Normally, they are turned off at 17:00 hours. These lights may have influenced spawning by acting as the “moonlight” cue.

The purpose of the skylight located in the room was only to add more light to the room during the day but not to influence timing of coral spawns. Larger or more skylights and better positioning of them may have allowed greater influence by natural moonlight. However, covering the skylight might have permitted the artificial moonlight source to be a stronger cue.

#### **Spawning behavior**

It seems that *A. valida*, in our tank, splits its spawn between two consecutive nights, given the observed and inferred spawn dates. Also, polyp dissections showed oocytes at different levels of maturity, suggesting that more than one spawn would occur. Therefore, it is probable that *A. valida* was responsible for the unobserved spawn of July 4, 1999. It is interesting that wild *Acropora valida* colonies at Abrolhos Island, Western Australia, also exhibited split spawning, in two consecutive nights (Babcock *et al.*, 1994).

In our tank spawning was observed to begin with polyps located on lower (older) branches with gradual spawning of polyps on higher



(younger) branches. This is not uncommon in other Acroporids as well as other genera of coral (Babcock, Delbeek and Richmond, pers. com.).

Axial polyps were observed spawning. In general, these polyps are thought to be reproductively immature (Wallace, 2000) because they are young and actively growing. Wallace (1985) states that gravid axial polyps are rare but have been seen in *A. valida*. It is possible that the axial polyps in our *A. valida* colony were sexually mature due to slow growth. A reduced growth rate may have been due to old light bulbs (one year and two months in operation at the time of spawning) or light levels (1999 spawn), or other general constraints on growth in captivity.

### **Fertilization experiments**

Although precise techniques such as those described in various papers for self-fertilizing Acroporids (Willis *et al.*, 1997) were not used, it is not surprising that our *A. valida* did not self-fertilize. Richmond (2000) reports that at least in some species of Acroporids self-fertilization is less likely than cross-fertilization.

### **CONCLUSION**

Even though pinpointing the exact night of a spawn was not possible for *A. valida*, it was possible to predict spawning within 10 days. Predicting the spawns of corals in captivity can offer a number of important opportunities. The spawning of *Montipora capitata* at the Waikiki Aquarium in Oahu, Hawaii, for example, is advertised to its membership for viewing experience and educational purposes (Waikiki Aquarium, 2001). The Maui Ocean Center in Maui, Hawaii also provides similar events based on predictable coral spawns of *M. capitata* (www1). Captive coral spawns also provide opportunities for propagation efforts. Projects such as SECORE (Petersen, 2005) where coral gametes are collected in the wild and fertilized in labs, may all take place within an aquarium institution if species can self-fertilize or multiple colonies of the same species spawn. Spawns in captivity may also provide researchers convenient access to study coral reproduction and to collect their gametes.

### **ACKNOWLEDGEMENTS**

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## PERSONAL COMMUNICATIONS

Babcock, R.C., 2007, CSIRO, Floreat, WA 6014 Australia

Delbeek, C., 2007, Waikiki Aquarium, Honolulu, HI 96815 USA

Fukami, H., 2007, Seto Marine Biological Laboratory, Kyoto University, Wakayama 649-2211 Japan.

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Knowlton, N., 2007, Scripps Institution of Oceanography (UCSD), La Jolla, California, 92093 USA.

Richmond, R.H., 2007, Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI 96813 USA.

Votava, P., 2007, Formerly of Sea Dwelling Creatures, Los Angeles, CA USA.

## INTERNET SITES

www1: <http://www.mauitime.com/vOiss2?news.html>

## APPENDIX I: Tank Specifications

Tank name	Birch Aquarium at Scripps
Location	La Jolla, California USA
SIZE AND VOLUME	
Size	2.56 m x 1.5 m x 1.2 m
Volume	4,620 L
Sump Volume	576 L
LIGHTING AND PHOTOPERIOD	
Lighting	4x 400 W Iwasaki (Iwasaki Electric Co. Ltd. Tokyo, Japan) metal halide bulbs (6,500 K), 2x 250 W Venture (Venture Lighting International, Ohio USA) metal halide bulbs (5,500 K), 2 pairs, 96 W compact fluorescent bulbs, each 6,700 K and 7,200 K (Panasonic Corporation of North America, New Jersey USA)
Photoperiod	07:30–19:30 (12 hours)
Artificial moonlight	From May, 1999 to April, 2002, an 85 W blue, incandescent floodlight turned on at 22:00 and off at 03:00 hours by plug-in timer. From May 2002–2003, a blue incandescent, floodlight was controlled by “Solar 1000 L1” (BlueLine Aquatics, USA) following the natural lunar phase
Skylight (opaque)	1 m x 1 m, 3.88 m high. Located in same room as tank, thus providing filtered, indirect sunlight and moonlight
FILTRATION AND CIRCULATION	
Circulation	~150 L.min <sup>-1</sup> via 3/4HP Jacuzzi Pump (Jacuzzi Bros. Division, Little Rock, AR USA)
Water flow	Through 3-way “True Blue” pneumatic valve (Plast-O-Matic Valves, Inc. Cedar Grove, NJ USA) into PVC spray bars located at both ends of tank. Valve was timed to switch water flow direction every three minutes
Bio-filtration	270 kg Solomon Island live rock
Mechanical filtration	Two, 1 m long, 100 µm filter socks
Foam fractionation	ETS 2400 Protein skimmer (A. E. Technology Inc.-06, Beacon, NY USA) with 1/2HP Jacuzzi Pump (Jacuzzi Bros. Division, Little Rock AR USA)
WATER CHANGES	
Water Source	Open system, receiving continual flow of 1–2 L.min <sup>-1</sup> , sand-filtered, natural seawater from Scripps Pier (La Jolla, CA)
Incoming water parameters	Salinity: 33.5 ppt (Walker <i>et al.</i> , 1994) pH: 8.0 alkalinity: 2.5 mEq.L <sup>-1</sup> Calcium: 400–410 mg Ca <sup>2+</sup> .L <sup>-1</sup> all tested with Salifert brand test kits (Salifert, Holland, Netherlands). Note: It is unknown whether the <i>A. valida</i> colony fed on the fine organic matter (plankton and detritus) that may have passed through the sand filters. If so, this may have been a benefit to the coral with respect to growth and energy resource allocation as it relates to reproduction.